

Expert Review

BCRP/*ABCG2* in the Placenta: Expression, Function and Regulation

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Received December 17, 2007; accepted January 8, 2008; published online January 18, 2008

Abstract. Knowledge concerning transport of maternally administered drugs across the placental barrier is essential for determining potential toxicity of drugs to the fetus and the value of drug therapy during pregnancy. An important determinant for fetal drug exposure is the expression of efflux transporters in the placenta. Among human tissues, the ATP-binding cassette efflux transporter BCRP (gene symbol *ABCG2*) is most abundantly expressed in the apical membrane of placental syncytiotrophoblasts. Although the precise physiological role of BCRP in the placenta is still unclear, existing data strongly suggest that BCRP plays an important role in protecting the fetus against the potential toxicity of drugs, xenobiotics, and metabolites by expelling them across the placental barrier. In this review, we summarize the current knowledge with respect to the expression, function, and polymorphisms of BCRP, as well as transcriptional and posttranscriptional regulation of the transporter in the placenta. Finally, clinical significance of BCRP in the placenta for drug therapy in pregnant women is discussed.

KEY WORDS: ABC transporter; BCRP/*ABCG2*; fetus; placenta; pregnancy.

INTRODUCTION

Pregnant women often need to take medication to treat diseases including viral, fungal, or bacteria infections, epilepsy, hypertension, or pregnancy-induced conditions such as depression, nausea and gestational diabetes. In a study in which 578 pregnant women were interviewed, researchers found that 95.8% of the participants took at least one prescription drug, 92.6% of the participants self-medicated with over-the-counter medications, and 45.2% of the participants used herbal medications during pregnancy (1). More recently, a multi-center study also showed that 64% of all pregnant women in the USA used at least one prescription drug other than a vitamin or mineral supplement in the 270 days before delivery (2). Moreover, a large proportion of pregnant women (5–10%) received FDA category D or X drugs which are potentially teratogens, and the general pattern of drug use has been with higher use in early pregnancy compared to later trimesters (2,3). Thus, a major concern arising from the use of medication by pregnant women is the transfer of drugs across the placental barrier, leading to potential toxicity to the developing fetus, particularly at early gestational stages.

Most maternally administered drugs can cross the placental barrier by passive diffusion to some extent which is primarily determined by the physicochemical properties (e.g., molecular weight, pK_a , and lipid solubility) and/or the

pharmacokinetic characteristics (e.g., elimination half-life in the mother, protein binding in fetal and maternal compartments, and metabolism in the placenta and fetus) of the drugs (4–6). In recent years, the importance of ATP-binding cassette (ABC) efflux transporters expressed in the apical membrane of placental syncytiotrophoblasts in moderating drug penetration across the placental barrier and thereby limiting fetal drug exposure has been appreciated (7,8). The first ABC transporter that has been recognized to play a significant role in protecting the fetus is P-glycoprotein (P-gp). For example, Lankas *et al.* (9) showed that the absence of P-gp expression in the placenta of pregnant mice significantly increased the toxicity of the pesticide avermectin to the developing fetus with enhanced placental penetration of the pesticide. The two other major ABC efflux transporters that are expressed in the apical membrane of placental syncytiotrophoblasts and may also be important in protecting the fetus are the multidrug resistance protein 2 (MRP2) and the breast cancer resistance protein (BCRP; Fig. 1). Expression of ABC transporters in the placenta in general has been reviewed recently (8,10,11).

This review focuses on BCRP. We will first give a brief overview of BCRP followed by the current knowledge regarding expression and function of the transporter in the placenta. Substantial variation in BCRP expression has been observed in human placenta (12), suggesting that considerable variability could exist in fetal exposure to drugs, xenobiotics, and metabolites. Such variable expression and/or activity may be caused by genetic polymorphisms of BCRP (12). BCRP expression in the placenta is possibly tightly controlled during pregnancy by pregnancy-related steroid hormones, growth factors, and cytokines. We therefore will

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also review the current understanding concerning single-nucleotide polymorphisms of BCRP in human placenta and the molecular mechanisms by which BCRP expression in the placenta is regulated. Finally, clinical significance of BCRP expression in the placenta will be discussed.

Breast Cancer Resistance Protein (BCRP)

BCRP is an approximately 75 kDa plasma membrane transporter belonging to the subfamily G of the large human ABC transporter superfamily. BCRP is the second member of the subfamily G and hence designated as *ABCG2*. BCRP was first cloned in 1998 from a breast cancer cell line, MCF-7/AdrVp which is highly resistant to doxorubicin with cross-resistance to daunorubicin and mitoxantrone (13). Shortly after that, an almost identical transporter termed MXR was discovered in a highly mitoxantrone-resistant human colon carcinoma cell line S1-M1-80 (14). Allikmets *et al.* (15) screened expressed sequence tag database and isolated ABCP cDNA that is essentially identical to the cDNA of BCRP or MXR. ABCP (ABC transporter in placenta) was so named to reflect its high level expression in human placenta. BCRP, MXR and ABCP are essentially the same protein with only a few amino acid differences. It has now been well established that BCRP is an ATP-dependent efflux transporter that is able to expel chemotherapeutic agents out of the cell, and is therefore considered as one of the most important ABC transporters that confer multidrug resistance in cancer cells (16,17).

Functional studies in the last decade indicate that BCRP can transport a broad spectrum of substrates, ranging from chemotherapeutic agents to organic anions. Substrate specificity of BCRP has been extensively reviewed (18,19). Typical chemotherapeutic agents that are transported by BCRP include mitoxantrone, camptothecin derivatives (e.g., topotecan and irinotecan), and anthracyclines (e.g., daunorubicin and doxorubicin; 16). Many of these anti-cancer drugs are also P-gp substrates. Nucleoside analogs such as zidovudine (AZT) and lamivudine (3TC) are also BCRP substrates (20,21). BCRP substrates also include, among others, prazosin (22), the tyrosine kinase inhibitors CI1033 and STI571 (23,24), dipyrindamole (25), and phytoestrogens (26). Of particular interest is that various drugs commonly administered to pregnant women, including nitrofurantoin (27), cimetidine (28) and glyburide (29), are BCRP substrates. Thus, a large number of drugs from various therapeutic categories, including anti-cancer drugs, antibiotics, anti-hypertensive, and anti-diabetics, have been identified as BCRP substrates. BCRP can also transport a wide variety of organic anions, conjugated or unconjugated, including estrone-3-sulfate, 17 β -estradiol 17-(β -D-glucuronide), dehydroepiandrosterone (30), and methotrexate (31). Collectively, BCRP displays an extremely broad spectrum of substrate specificity that is overlapping, but distinct from that of P-gp or MRP2. In general, P-gp preferentially transports uncharged hydrophobic compounds and some weakly basic substances (32); however, BCRP can transport both hydrophobic and hydrophilic substrates which are conjugated or unconjugated (16,18,32). MRP2 mainly transports organic anions, and glutathione, sulfate, and glucuronate conjugates (33). While BCRP can transport both sulfate and glucuronide conjugates, sulfate conjugates seem to

be the preferred substrates (34). However, MRP2 seems to preferentially transport glutathione conjugates (with lower K_m values; 33), and BCRP has so far not been shown to transport glutathione conjugates.

A variety of BCRP inhibitors have already been identified, including, among others, GF120918 (a second-generation P-gp inhibitor which belongs to the acridone carboxamide derivatives; 35), fumitremorgin C (FTC; a natural product secreted from the fungi *Aspergillus fumigatus*) and its analogs (36), tyrosine kinase inhibitors (e.g., CI1033 and STI571; 37,38), anti-HIV protease inhibitors (e.g., nelfinavir and ritonavir; 39), calcium channel blockers (25), immunosuppressants (40), and a variety of food dietary flavonoids (41). More detailed information on BCRP inhibitors can be found in recent reviews (18,42). Identification of BCRP substrates and inhibitors provides the opportunity to pharmacologically modulate BCRP function as a tool of drug therapy (e.g., circumvention of drug resistance in cancers) and allows understanding the molecular mechanisms underlying potential BCRP-mediated drug-drug interactions.

Tissues Expression and Membrane Localization of BCRP

BCRP expression in human tissues has been extensively investigated. Doyle *et al.* (13) assessed BCRP mRNA expression using commercially prepared human multi-tissue Northern blots. The highest level of BCRP mRNA was seen in placenta, which was approximately 100 times greater than that in liver, small intestine, colon, brain, liver, ovary, testis, or prostate. BCRP mRNA levels were also high in liver and small intestine. There was little or no BCRP mRNA expression in heart, lung, skeletal muscle, kidney, pancreas, spleen, thymus, or peripheral-blood leukocytes. BCRP was named ABCP because of its extremely high level expression in the placenta compared with other tissues (15). Subsequently, the immunohistochemical study by Maliepaard *et al.* with two different BCRP-specific monoclonal antibodies revealed that BCRP protein was most strongly expressed in the colon epithelium, the placental syncytiotrophoblasts, the small intestinal epithelium, liver canaliculi, and blood vessel capillaries in the brain (43). BCRP protein levels in other tissues were low. In general, the pattern of BCRP protein expression in human tissues determined by immunohistochemistry matches well with that of BCRP mRNA expression obtained in Northern blotting studies. BCRP expression in normal tissues may play a protective role for these tissues. The tissue distribution pattern of Bcrp1, the murine homolog of human BCRP, highly resembles that of human protein with substantial Bcrp1 mRNA and protein expression in the placenta, small intestine, and liver, except that there is little expression of BCRP in human kidney whereas Bcrp1 expression in mouse kidney is substantial (44-46). BCRP gene has also been cloned in rat (46,47), rhesus macaque (48), and porcine (49). The tissue distribution pattern of BCRP in rhesus macaque and porcine has yet to be determined.

With respect to membrane localization of BCRP in human tissues, Maliepaard *et al.* (43) showed that BCRP is primarily expressed in the apical membrane of the placental syncytiotrophoblasts, in the apical membrane of the epithelium in small intestine and colon, and in the liver canalicular membrane. BCRP is also highly expressed at the luminal

surface of the microvessel endothelium of human brain (50). The substantial expression of BCRP in organs important for drug disposition (e.g., small intestine, liver, blood-brain barrier, and placenta) implies that BCRP could play a significant role in the absorption, distribution, and elimination of drugs that are BCRP substrates. Indeed, the importance of BCRP in drug disposition has now been demonstrated in numerous studies (51–54). The pharmacological significance of BCRP in drug transport has been extensively reviewed elsewhere (18,19,27,35,55–57). The apical side localization of human BCRP in placental syncytiotrophoblasts has been confirmed in various studies (58–61). We have shown that mouse Bcrp1 is also localized in the apical membrane of placental syncytiotrophoblasts of the pregnant mouse (45). Similarly, rat Bcrp has been shown to be strongly expressed in the rat placental labyrinth zone in which maternal blood is separated from fetal blood vessels by trophoblasts (62). A schematic representation of BCRP localization in the placenta is shown in Fig. 1.

Gestational Change of BCRP Expression in the Placenta

Recent studies indicate that BCRP expression in the placenta changes with gestational age. Meyer zu Schwabedissen *et al.* demonstrated that the BCRP mRNA levels in human placenta at preterm (28 ± 1 weeks, 15 placenta) were approximately two times greater ($p < 0.05$) than that at term (39 ± 2 weeks, 29 placenta), and BCRP protein expression showed the same pattern as that of BCRP mRNA (59). These authors did not measure BCRP expression in human placenta

earlier than 28 weeks. However, another study with smaller sample size (six to eight placenta at each gestational age) did not show significant change in BCRP mRNA levels in human placenta with advancing gestation, but BCRP protein levels slightly increased towards the end of gestation (61). Mathias *et al.* also reported that the BCRP protein and mRNA levels in human placenta did not significantly change with gestational ages, but again these data were obtained with a limited sample size (four to six placenta at each gestational age) with substantial variations (63). The reason for this apparent discrepancy remains to be investigated. The data from rodent studies seem to be consistent with those reported by Meyer zu Schwabedissen *et al.* (59). In rat, Yasuda *et al.* found that rat Bcrp protein levels in the placenta at gestation day 14 were significantly higher than those at gestation day 20 (term in rat is approximately 21 days; 64). Likewise, our laboratory also demonstrated that Bcrp1 expression (protein and mRNA) in the placenta of pregnant mice peaked at gestation day 15 (term in mice is approximately 20–21 days; 45). Kalabis *et al.* reported that Bcrp1 mRNA levels in the placenta of pregnant mice decreased progressively from gestation day 9.5 toward term; however, Bcrp1 protein expression did not change significantly during gestation (65). Overall, BCRP/Bcrp1 expression in the placenta may be under tight control during pregnancy. Taken together, the substantial expression in the apical membrane of placental syncytiotrophoblasts indicate that BCRP/Bcrp1 may play an important role in protecting the fetus by expelling drugs, xenobiotics, and metabolites across the placental barrier, particularly at mid-gestational ages. We note that, while

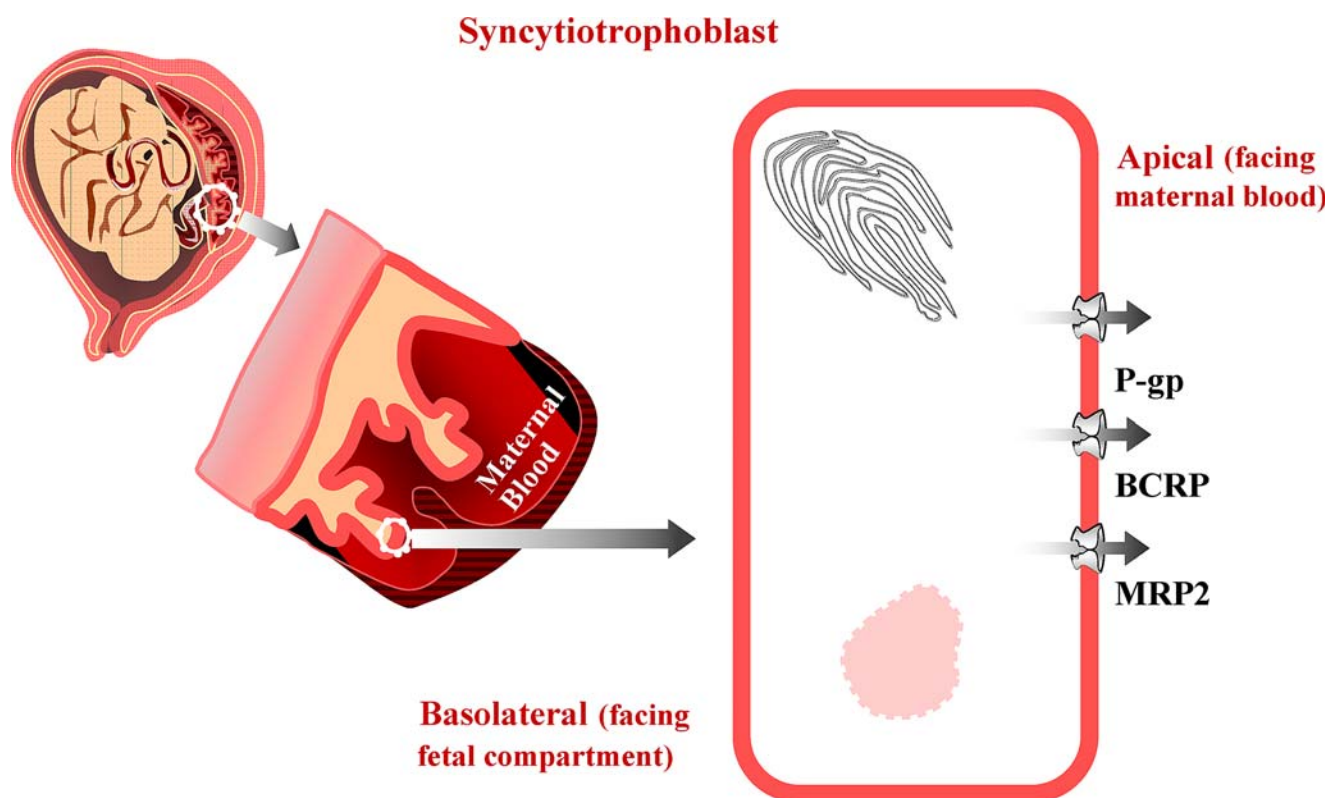


Fig. 1. A schematic representation of localization of the major ABC efflux transporters, P-gp, BCRP, and MRP2, in the apical membrane of the placental syncytiotrophoblast. *P-gp* P-glycoprotein (*ABCB1*), *BCRP* breast cancer resistance protein (*ABCG2*), *MRP2* multidrug resistance protein 2 (*ABCC2*)

BCRP expression in the placenta peaks at mid-gestation as discussed above, P-gp (63,66) and MRP2 (67) expression in the placenta progressively decreases and increases, respectively, with gestational age towards term. It is possible that the differential placental expression of BCRP, P-gp, and MRP2 over the course of pregnancy provides a compensatory mechanism for protection of the fetus at different gestational stages.

Transport Function of BCRP in the Placenta

The activity of BCRP/Bcrp1 in placental transport has been analyzed in studies using *in vitro* cell culture, *ex vivo* perfused placenta, and *in vivo* animal models. The data published thus far are summarized in Table I. Kolwankar *et al.* (68) reported BCRP-mediated mitoxantrone transport in microvillus membrane vesicles isolated from human term placenta. Most recently, Gedeon *et al.* (69) showed that uptake of glyburide into the inside-out membrane vesicles isolated from human term placenta was significantly increased by the BCRP inhibitor novobiocin, but not by the P-gp inhibitor verapamil and the MRP inhibitor indomethacin, suggesting that glyburide was transported by BCRP. Transport activity of BCRP has also been illustrated *in vitro* with the “model” human placental BeWo cell line or primary trophoblasts in various studies using mitoxantrone or Hoechst 33342 as a BCRP substrate (70–72). Staud *et al.* (62) examined the transport activity of Bcrp1 in perfused rat placenta using cimetidine as a Bcrp1 substrate. Rat Bcrp1 was shown to significantly limit the maternal-to-fetal transport of cimetidine. When fetal perfusate was recirculated, rat Bcrp1 could actively transport cimetidine from the fetal to the maternal compartment against a concentration gradient. The first study demonstrating *in vivo* transport activity of Bcrp1 in the placenta was performed in P-gp-knockout pregnant mice (51). Using P-gp-deficient pregnant mice, Jonker *et al.* (51) demonstrated that co-administration of the Bcrp1 inhibitor GF120918 increased the fetal concentration of topotecan (a BCRP/Bcrp1 substrate) twofold at 30 min after drug administration in the pregnant mice, compared with that in the pregnant vehicle-treated control animals. *In vivo* studies demonstrating transport activity of placental Bcrp1 using Bcrp1-knockout mice have been reported for topotecan and phytoestrogens (26,44). These studies demonstrated increased fetal/maternal plasma concentration ratios in Bcrp1-knockout pregnant mice compared with those in wild-type pregnant mice, at only one time point after drug administration as a measure of fetal drug exposure. Most recently, our laboratory, for the first time, reported detailed transplacental pharmacokinetics of a model BCRP/Bcrp1 substrate, nitrofurantoin, in the pregnant mouse, and examined the role of Bcrp1 in determining fetal exposure of nitrofurantoin (73). We measured the maternal plasma and fetal AUCs of nitrofurantoin in pregnant mice after drug administration by retro-orbital injection. After 60 min of drug administration, nitrofurantoin in the systemic circulation was nearly completely eliminated in both the wild-type and Bcrp1-knockout pregnant mice. While the maternal plasma AUC of nitrofurantoin was only slightly increased in Bcrp1-knockout pregnant mice compared with that in wild-type pregnant mice, the fetal AUC in Bcrp1-knockout pregnant mice was

approximately 5 times greater than that in wild-type pregnant mice. These results clearly suggest that Bcrp1 significantly limits fetal distribution of nitrofurantoin in the pregnant mouse. Such *in vivo* transplacental pharmacokinetic studies in animal models are particularly valuable, as similar studies of direct placental drug transport in humans are not feasible due to ethical reasons.

We also performed similar *in vivo* transplacental pharmacokinetic studies for glyburide, an antidiabetic drug commonly used to treat gestational diabetes. We found that while the maternal plasma AUCs of glyburide in the wild-type and Bcrp1-knockout pregnant mice were comparable, the fetal AUC of glyburide in the Bcrp1-knockout pregnant mice was two times greater than that in the wild-type pregnant mice (74). Recently, Kraemer *et al.* (75) performed *ex vivo* perfusion studies of human placenta to quantify placental transfer of glyburide. These authors have excluded albumin from the perfusion buffer so that net transfer of glyburide could be measured without the effect of high protein binding. Substantial fetal-to-maternal transfer of glyburide against a concentration gradient was observed. Furthermore, the transfer of glyburide across the placental barrier could not be inhibited the addition of verapamil, a P-gp inhibitor. These data suggest that glyburide was possibly effluxed by a transporter other than P-gp or P-gp is not a major player in the glyburide transport by human term placenta. We have previously shown that verapamil is not an effective inhibitor for BCRP (25). Thus, BCRP is likely a major player in the efflux of glyburide by human term placenta. The results of our animal studies provided further evidence to support this conclusion. However, since glyburide is also a P-gp substrate (76) and verapamil used in the study of Kraemer *et al.* is a relatively weak P-gp inhibitor, the role of P-gp in placental transport of glyburide cannot be excluded. Nevertheless, since it has been shown that the BCRP mRNA level was approximately ten times greater than that of P-gp in human term placenta (70), it is reasonable to assume that BCRP likely plays a much greater role than P-gp in transport of glyburide by human term placenta. High plasma protein binding may also contribute to the minimal fetal distribution of glyburide *in vivo* (77). Taken together, the *in vitro* transport, *ex vivo* perfusion, and *in vivo* transplacental pharmacokinetic studies all point to the unique role of BCRP/Bcrp1 in limiting fetal exposure of glyburide.

Physiological Role of BCRP in the Placenta

Although it is now quite clear that BCRP plays an important role in protecting the fetus against potential toxicity of drugs and xenobiotics, few studies have been done to demonstrate the physiological role of BCRP in the placenta. Placenta is the organ involved in the synthesis of steroid hormones such as progesterone and estrogens during pregnancy. Dehydroepiandrosterone (DHEA) and its sulfate conjugate (DHEAS) are important precursor molecules for placental estrogen synthesis. Estrone-3-sulfate (E3S) is a metabolite of estrogens but can function as a pool of inactive estrogens. Both DHEAS and E3S are effective substrates of BCRP (30,60); therefore, BCRP in human placenta is likely involved in the elimination of these endogenous sulfate conjugates from the fetal compartment into the maternal

Table 1. Transport Function of Placental BCRP/Bcrp1

Substrate	Transport System Used	Comment on Function	Reference
MX	Membrane vesicles isolated from human term placenta	MX uptake into the vesicles was inhibited by FTC	(68)
Glyburide	Membrane vesicles isolated from human term placenta	Glyburide uptake into the vesicles was inhibited by novobiocin	(69)
MX	BeWo cells	Intracellular MX accumulation was increased by GF120918 and Ko143	(70)
Hoechst	BeWo cells, Jar cells, and primary trophoblasts	Intracellular Hoechst accumulation was increased by FTC	(71)
MX	BeWo cells	Intracellular MX accumulation was increased by FTC	(72)
Cimetidine	Perfusion with rat placenta	Active transport of cimetidine from the fetal to the maternal compartment against a concentration gradient was shown	(62)
Topotecan	P-gp-deficient pregnant mice	The fetal/maternal plasma concentration ratio of topotecan in P-gp-deficient mice treated with GF120918 was increased twofold compared with that in the vehicle-treatment controls	(51)
Topotecan	Bcrp1 ^{-/-} pregnant mice	The fetal/maternal plasma concentration ratio of topotecan in Bcrp1 ^{-/-} mice was increased twofold compared with that in wild-type animals	(44)
Genistein	Bcrp1 ^{-/-} pregnant mice	The fetal/maternal plasma concentration ratio of genistein in Bcrp1 ^{-/-} mice was increased twofold compared with that in wild-type animals	(26)
NFT	Bcrp1 ^{-/-} pregnant mice	The fetal/maternal plasma AUC ratio of NFT in Bcrp1 ^{-/-} mice was increased fivefold compared with that in wild-type animals	(73)
Glyburide	Bcrp1 ^{-/-} pregnant mice	The fetal/maternal plasma AUC ratio of glyburide in Bcrp1 ^{-/-} mice was increased 2.5-fold compared with that in wild-type animals	(74)
Glyburide	Perfusion with human term placenta	The active transport of glyburide from the fetal to the maternal circulation against a concentration gradient was not significantly affected by the P-gp inhibitor verapamil	(75)

MX Mitoxantrone, Hoechst Hoechst 33342, NFT nitrofurantoin, FTC fumitremorgin C

circulation. Thus, it has been suggested that BCRP may regulate placental estrogen synthesis through modulating intracellular DHEAS and E3S concentrations (60).

Recently, Evseenko *et al.* (58,78) showed that inhibition of BCRP activity by a BCRP-specific inhibitor Ko143 increased cytokine-induced apoptosis in primary trophoblasts and BeWo cells. Decreasing BCRP expression in BeWo cells by transfection of BCRP siRNA significantly increased the cell sensitivity to cytokine- and exogenous C6 and C8 ceramide-induced apoptosis. Silencing of BCRP expression also increased intracellular ceramide levels after cytokine exposure, but did not affect cellular protoporphyrin IX concentrations or sensitivity to activators of intrinsic apoptosis pathway. These authors further demonstrated that BCRP expression in placenta from pregnancies with idiopathic fetal growth restriction (IUGR) was significantly lower than that from normal pregnancies. Based on these data, the authors proposed that BCRP may play a hitherto unrecognized role in the placenta, protecting trophoblasts against apoptosis induced by cytokines or other activators via modulation of ceramide signaling, and BCRP is likely a survival factor in differentiation of placental trophoblasts. These data support the hypothesis that decreased BCRP expression in the placenta from IUGR pregnancies may result in placental function deficit, thus contributing to fetal growth restriction. Further studies are needed to confirm this hypothesis.

Genetic Polymorphisms of BCRP in the Placenta

Several laboratories have reported naturally occurring variants of BCRP. Notably, the single nucleotide polymorphisms (SNPs) G34A and C421A, resulting in alterations of BCRP protein at position 12 (V12M) and 141 (Q141K), respectively, occur at a relatively high frequency in most ethnic populations. For example, in the Japanese population, 39–50% are heterozygous and 7% are homozygous for C421A (79, 80). In the Chinese population, 60% are heterozygous for C421A (80). Other BCRP SNPs including A616C and A1768T are much less frequent with allele frequencies less than 1% (79–82). In addition, a polymorphism that results in a substitution of stop codon for Gln at position 126 has been identified (79). BCRP SNPs may result in variable expression of the transporter in tissues. Recently, Kobayashi *et al.* (12) examined the relationship between BCRP gene polymorphisms and protein expression in human placenta of the Japanese population. They showed that G34A and C421A occur at allele frequencies of 18% and 35%, respectively. Also, C376T which results in a stop codon at position 126 occurs at <1% frequency. This is consistent with the data reported by others (79,80). Kobayashi *et al.* also identified various SNPs with low allele frequencies (<1%), such as G1322A and T1465C, in placenta of the Japanese population, which were not reported by others. The study by Imai *et al.* (79) showed that the C421A polymorphism was associated with significantly lower expression and activity of the Q141K variant compared with the wild-type protein. Similarly, in placenta of the Japanese population, Kobayashi *et al.* illustrated that the mean BCRP protein level of the A421 homozygotes was approximately 50% of that of the C421 allele, and heterozygotes displayed an intermediate level. They further showed that this difference is likely

caused by posttranscriptional regulation rather than changes in mRNA expression because polymorphism-dependent changes in BCRP mRNA expression were not observed (12). These data suggest that the C421A polymorphism could potentially decrease BCRP expression and activity in human placenta, leading to increased fetal drug exposure.

In vitro Regulation of BCRP Expression in Placental Cells

Pregnancy is one of the major physiologically stressful events, during which hormone concentrations are drastically changed. Therefore, it is not surprising that the expression of drug transporters and metabolic enzymes in organs important for drug disposition may be altered during pregnancy. As mentioned above, several laboratories have demonstrated that the expression of human BCRP as well as mouse and rat Bcrp1 in the placenta is gestational age-dependent (45,59,64). Likewise, Bcrp1 protein levels in the liver and kidney of pregnant mice reached a maximum at gestation day 15, but there was no significant change in Bcrp1 protein levels in small intestine over gestational age (45). At present, no data are available regarding the effect of pregnancy on BCRP/Bcrp1 expression in the liver, kidney, and small intestine of species other than mice.

The molecular mechanisms by which pregnancy affects BCRP expression in the placenta and other organs is yet unknown. The promoter of human *BCRP* gene has been cloned (83), permitting studies on regulation of *BCRP* gene expression at the transcriptional level. The *BCRP* promoter lacks a TATA-box and contains several putative Sp sites which are downstream from a putative CpG island. Whether Sp nuclear transcriptional factors are involved in the control of constitutive expression of the *BCRP* gene is not known. Recently, other nuclear receptors such as peroxisome proliferators-activated receptor gamma (PPAR γ ; 84), hypoxia-inducible factor 1 (HIF-1; 85), and aryl hydrocarbon receptor (AhR; 86,87) have been shown to be involved in the induction of *BCRP* gene transcription. Factors other than nuclear receptors have also been reported to affect BCRP expression. For example, To *et al.* (88) illustrated that aberrant methylation in the predicted CpG island in the *BCRP* promoter region suppresses transcription of the *BCRP* gene. Alternative promoter usage has also been demonstrated to cause differential expression of *BCRP* mRNA in drug-resistant cells and normal tissues (89,90). Whether the above mentioned regulation pathways possibly play a role in the control of BCRP expression in the placenta is yet to be determined.

The concentrations of pregnancy-related hormones such as progesterone and 17 β -estradiol rise significantly throughout pregnancy (91). Therefore, it is reasonable to hypothesize that BCRP expression in the placenta may be increased in association with increased concentrations of these hormones. We have recently shown that progesterone receptor isoforms, PRA and PRB, differentially regulate expression of BCRP in the model human placental cell line, BeWo (92). We found that progesterone up-regulated BCRP expression in BeWo cells through PRB, but not PRA. In addition, PRA repressed the PRB activity. We also identified a progesterone response element (PRE) in the *BCRP* promoter region (92). Evseenko *et al.* reported that progesterone treatment did not affect

BCRP expression in primary trophoblasts (93). It is possible that the trophoblasts secrete a high level of endogenous progesterone by which the progesterone receptors are saturated; therefore, further addition of exogenous progesterone could not have an effect. We observed this phenomenon in BeWo cells (92). Progesterone is highly produced by the placenta after weeks 10 in pregnancy (94). Also, progesterone receptor expression has been demonstrated in human placenta (95). Thus, placenta is very likely a target tissue for the action of progesterone. It is therefore reasonable to hypothesize that progesterone may induce BCRP expression in the placenta through PRB and augment the protective role of the transporter during pregnancy. We will test this hypothesis in future study. Kalabis *et al.* examined the effect of progesterone on Bcrp1 expression in the placenta of pregnant mice, and found that the daily progesterone treatment starting at gestation day 14.5 until gestation day 18.5 significantly increased maternal progesterone concentrations, but did not affect Bcrp1 mRNA and protein expression in the placenta at gestation day 18.5 as compared with vehicle-treatment controls (65). It has been shown that the maternal plasma concentrations of endogenous progesterone at gestation day 14.5 were approximately 60 ng/ml (~190 nM; 96) and the progesterone concentrations in placenta tissues could be even greater. The progesterone receptors have possibly already been saturated at such high progesterone concentrations. Moreover, we have shown that the progesterone receptor expression in the placenta of pregnant mice is significantly decreased from gestation day 10 towards term (term in mice is approximately 20–21 days; 45). Therefore, an induction of placental Bcrp1 expression by additional exogenous progesterone from gestation day 14.5 to gestation day 18.5 may not occur. Collectively, the possibility that progesterone regulates BCRP/Bcrp1 expression in the placenta *in vivo* over the course of pregnancy warrants further investigation.

The effect of 17 β -estradiol on BCRP expression is conflicting. It has been shown that 17 β -estradiol induces BCRP expression in various cancer cell lines including the BeWo cells via estrogen receptor α (ER α), and an estrogen response element (ERE) in the *BCRP* promoter region has also been identified (97,98). In contrast, Imai *et al.* reported that 17 β -estradiol decreased BCRP expression in ER α -positive cancer cell lines through posttranscriptional regulation (e.g., decreased protein biosynthesis and maturation; 99). As to why the posttranscriptional down-regulation of BCRP by 17 β -estradiol requires the presence of a nuclear receptor ER α is not known. We also found that 17 β -estradiol down-regulated BCRP expression in BeWo cells (72). The reason for this apparent discrepancy remains to be determined. We note that the PRE in the *BCRP* promoter region is exactly the same as the ERE published by Ee *et al.* (97). That progesterone and estrogen receptors share the same or similar response elements is possible, as earlier studies suggest that the regulatory elements for different steroids, including progesterone, 17 β -estradiol, and glucocorticoids, are either similar or at least share structural features (100). Thus, progesterone and 17 β -estradiol could make an impact on each other in regulation of BCRP, when the two hormones are combined. The real situation could be very complex, as 17 β -estradiol by itself can induce PRB

expression (72,101) and down-regulate BCRP expression (99), and on the other hand, PRA can repress the estrogen receptor activity (102). In BeWo cells, we showed that the 17 β -estradiol treatment alone down-regulated BCRP expression (72), presumably due to posttranscriptional regulation as demonstrated by Imai *et al.* (99); however, the combined treatment of BeWo cells with 17 β -estradiol and progesterone significantly increased BCRP expression compared with progesterone treatment alone (72). We have hypothesized that this combined effect is likely due to induction by 17 β -estradiol of PRB in BeWo cells which then induces BCRP expression through progesterone (72). The results that progesterone can up-regulate BCRP expression support this hypothesis. We noted that the combined effect of 17 β -estradiol and progesterone on BCRP expression in BeWo cells was dependent on concentrations of the two hormones used. For example, the inductive effect of progesterone on BCRP expression was inhibited when progesterone was combined with a high concentration of 17 β -estradiol (72). This is likely due to the effect of down-regulation on BCRP expression by 17 β -estradiol at a high concentration which overrides the inductive effect of progesterone.

We also observed that estradiol, human placental lactogen, and human prolactin could also induce BCRP expression in BeWo cells at physiological concentrations (103). Further studies are needed to elucidate the molecular mechanisms by which estradiol, placental lactogen, and prolactin up-regulate BCRP expression. Testosterone by itself did not affect BCRP expression at physiological concentrations. However, testosterone together with 17 β -estradiol increased BCRP expression, and this induction was abolished by ER α antagonist ICI-182,780 or the testosterone receptor antagonist flutamide or knock-down of ER α (103). Further analysis revealed that 17 β -estradiol increased testosterone receptor mRNA approximately 5.9-fold, suggesting that testosterone in combination with 17 β -estradiol increases BCRP expression, possibly through 17 β -estradiol-mediated up-regulation of the testosterone receptor.

BCRP expression in the placenta can also be influenced by cytokines and growth factors. Evseenko *et al.* showed that treatment of primary term trophoblasts with tumor necrosis factor- α (TNF- α) and interleukin-1 β (IL-1 β) significantly decreased BCRP protein and mRNA expression, but IL-6 had no significant effect (93). On the other hand, epidermal growth factor (EGF) and insulin-like growth factor II significantly increased BCRP protein and mRNA expression (59,93). There is evidence that the concentrations of cytokines such as TNF- α and IL-1 β are elevated in some of obstetric disorders such as preeclampsia and gestational diabetes. Thus, the fetal protective function of BCRP against xenobiotics may be decreased in such obstetric disorders. BCRP expression in primary trophoblasts was shown to be increased with trophoblast differentiation (71), which is consistent with the role of EGF that promotes trophoblast differentiation (104). Maternal inflammation could also alter BCRP expression in the placenta. For example, down-regulation of Bcrp1 expression in the placenta of endotoxin-treated near term pregnant rats has recently been indicated (11). The data regarding regulation of BCRP expression as discussed above are summarized in Table II.

Table II. Summary of Effects on BCRP Expression

Ligand	Nuclear Receptor or Other Factor	Effect	Reference
	PPAR γ	↑	(84)
	HIF-1	↑	(85)
	Aberrant methylation in CpG island of the <i>BCRP</i> promoter	↓	(88)
	Alternative promoter usage	Differential expression	(89,90)
Benzo[a]pyrene, Benzo[k]fluoranthene	AhR	↑	(86)
Curcumin, Resveratrol	AhR	↑	(87)
Progesterone	PRB	↑	(72, 92)
17 β -Estradiol	ER α	↑	(97, 98)
17 β -Estradiol	Posttranscriptional regulation	↓	(72, 99)
Estriol		↑	(103)
Human placental lactogen		↑	(103)
Human prolactin		↑	(103)
Testosterone	TR	↑	(103)
TNF- α , IL-1 β		↓	(93)
EGF	EGFR	↑	(59, 93)
Endotoxin		↓	(11)

PPAR γ Peroxisome proliferators-activated receptor gamma, HIF-1 hypoxia-inducible factor 1, AhR aryl hydrocarbon receptor, PRB progesterone receptor B, ER α estrogen receptor α , TR testosterone receptor, TNF- α tumor necrosis factor α , IL-1 β interleukin 1 β , EGF epidermal growth factor, EGFR epidermal growth factor receptor

Clinical Significance of BCRP Expression in the Placenta and Future Direction

The findings that BCRP plays a significant role in limiting fetal distribution of various drugs (Table I) have important clinical implications. To limit fetal drug exposure, drugs that are actively transported by BCRP may be preferred. On the other hand, if the fetus is the target of drug therapy, drugs that bypass BCRP in human placenta may be optimal. Glyburide is an excellent example of drugs that bypass BCRP-mediated transport by human placenta. Glyburide has been shown to be safe in pregnancy due to minimal fetal exposure (105). We and others have demonstrated that BCRP-mediated placental transport of glyburide is at least one of the mechanisms by which fetal exposure of the drug is limited (69,74). Many drugs are routinely used by pregnant women either intentionally or by misuse; however, for a majority of these drugs, it is still not known if they are substrates of BCRP and other efflux transporters. Much more work is therefore needed in the future to identify substrate drugs of BCRP and other efflux transporters in the placenta, and to determine how high their affinity is to these transporters. Placental drug transport has so far been primarily demonstrated in *in vitro* transport (placental cell lines or placenta membrane vesicles) or *ex vivo* placenta perfusion studies. The data obtained from such *in vitro* and *ex vivo* studies may not be used to accurately predict what happens *in vivo*. Thus, in addition to studies using improved *in vitro* models (e.g., MDCK cells stably expressing BCRP for Transwell transport studies to identify BCRP substrate drugs), more *in vivo* transplacental pharmacokinetic studies of drugs in animal models are needed in future work to obtain detailed information of fetal drug distribution kinetics during pregnancy. Such information is extremely valuable for drug therapy in pregnancy. Drug-drug interactions at the placental barrier through inhibition of BCRP can occur if a BCRP substrate drug happens to be co-administered with a BCRP inhibitor. Such interactions could lead to increased fetal drug

exposure through inhibition of BCRP in the placenta. Thus, caution should be taken when a substrate and an inhibitor of BCRP are concomitantly administered to pregnant women.

Another important area related to BCRP-mediated placental drug transport is the regulation of BCRP during pregnancy. Recent studies indicate that BCRP expression in placental cell lines or primary trophoblasts is affected by pregnancy-related steroid hormones, cytokines, and growth factors. Such findings have significant clinical relevance. For example, progesterone may induce BCRP expression in the placenta and augment the protective role of the transporter during pregnancy. On the other hand, patients with obstetric disorders associated with elevated cytokine levels may be at increased risk to fetal exposure of BCRP substrate drugs due to down-regulation of BCRP expression in the placenta by cytokines. Much more work is needed to elucidate the molecular mechanisms by which BCRP expression is regulated by pregnancy-related steroids, cytokines, and growth factors, and to determine if such regulation pathways contribute to gestational age-dependent BCRP/Bcrp1 expression in the placenta *in vivo*. In addition, the physiological role of BCRP in the placenta and the impact of BCRP SNPs on fetal drug exposure warrant further investigation.

In summary, significant progress has been made in the past 5 years with respect to the role of BCRP in placental drug transport. Understanding the molecular mechanisms of drug transport across the placental barrier as well as regulation of transporter expression in the placenta can help develop strategies to control fetal drug exposure and optimize drug therapy during pregnancy.

ACKNOWLEDGEMENT

The author gratefully acknowledges financial support from NIH grants HD044404 and HD047892. The author also greatly thanks Drs. Jashvant D. Unadkat (Department of Pharmaceutics, University of Washington) and Mary F. Hebert (Department of Pharmacy, University of Washington)

for collaborations on pregnancy-related studies. Mr. Hsiao Peng (Department of Pharmaceutics, University of Washington) is greatly appreciated for preparing the graphic.

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